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Increased Carotid Atherosclerosis in Andropausal Middle-Aged Men

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OBJECTIVES This study examined the association between carotid artery intima-media thickness (IMT), serum sex hormone levels, and andropausal symptoms in middle-aged men.

BACKGROUND Male sex hormones may play a dual role in the pathogenesis of atherosclerosis in men by carrying both proatherogenic and atheroprotective effects.

METHODS We studied 239 40- to 70-year-old men (mean ± SD: 57 ± 8 years) who participated in the Turku Aging Male Study and underwent serum lipid and sex hormone measurements. Ninety-nine men (age 58 ± 7 years) were considered andropausal (i.e., serum testosterone <9.8 nmol/l or luteinizing hormone [LH] >6.0 U/l and testosterone in the normal range), and in both situations, they had subjective symptoms of andropause (a high symptom score in questionnaire). Three were excluded because of diabetes. The rest of the men (age 57 ± 8 years) served as controls. Carotid IMT was determined using high-resolution B-mode ultrasound, and serum testosterone, estradiol (E_2), LH, and sex hormone-binding globulin were measured using standard immunoassays.

RESULTS Andropausal men had a higher maximal IMT compared with controls in the common carotid (1.08 ± 0.34 vs. 1.00 ± 0.23, $p < 0.05$) and in the carotid bulb (1.44 ± 0.48 vs. 1.27 ± 0.35, $p = 0.003$). Common carotid IMT correlated inversely with serum testosterone ($p = 0.003$) and directly with LH ($p = 0.006$) in multivariate models adjusted for age, total cholesterol, body mass index, blood pressure, and smoking.

CONCLUSIONS Middle-aged men with symptoms of andropause, together with absolute or compensated (as reflected by high normal to elevated LH) testosterone deficiency, show increased carotid IMT. These data suggest that normal testosterone levels may offer protection against the development of atherosclerosis in middle-aged men. (J Am Coll Cardiol 2005;45:1603–8)

Cross-sectional observational studies have demonstrated an inverse relationship between endogenous serum testosterone (T) levels and coronary heart disease (CHD) in males (1).

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Moreover, a decreased serum T concentration has been shown to be associated with acute ischemic strokes, infarct size, and mortality (2). Nevertheless, the independent prognostic value of T for the occurrence of future adverse cardiovascular events has not been shown (3,4), and the results from experimental studies are contradictory. In a recent series of human in vitro studies, T and other androgens were shown to exert several proatherogenic actions in males, including increased monocyte adhesion to endothelial cells (5), increased expression of endothelial adhesion molecules (5), accelerated foam cell formation (6), and upregulation of atherosclerosis-related genes (7). Other experimental studies, however, have demonstrated that T supplementation protects from atherosclerosis in castrated cholesterol-fed male rabbits (8) and in low-density lipoprotein (LDL) receptor-deficient male mice (9). In addition, the antiatherogenic effects of androgens seem to be inhibited by co-administration of aromatase inhibitors, implicating a critical role for the conversion of administered T to estradiol (E_2) (9).

In contrast to climacterium in women characterized by a more rapid and extensive decrease in ovarian function, the corresponding decline in androgen levels in men occurs more gradually and variably during aging. This condition is termed "partial androgen deficiency of the aging male," or simply "andropause." Only about 20% of 60- to 80-year-old men display genuine hypogonadism (10). There is currently no widely accepted normal range for serum T for aging men, and most previous studies have used cut-off values defined for young adult men. The threshold value for serum total T used to define hypogonadism has varied in previous studies,

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Abbreviations and Acronyms

AMS = Aging Male Symptoms Questionnaire
CHD = coronary heart disease
CV = coefficient of variation
E_2 = estradiol
HbA_{1c} = glycated hemoglobin
IMT = intima-media thickness
LDL = low-density lipoprotein
LH = luteinizing hormone
T3Q = Turku 3-Query

but most have used a concentration limit of <10 nmol/l, whereas the so far hypothetical condition of subclinical hypogonadism has been defined as elevated or high-normal luteinizing hormone (LH) >6 U/l, together with T in the low-normal range (11).

Carotid intima-media thickness (IMT) is a widely accepted noninvasive measure of preclinical atherosclerosis and an independent predictor of future adverse cardiovascular and cerebrovascular events (12–15). A limited number of previous observations have suggested that carotid IMT may be inversely associated with serum T levels in very elderly men (16), in men with type II diabetes (17), and in obese men (18). In a recent study, the serum T concentration was inversely associated with progression of carotid atherosclerosis in elderly men (19). The relationship between serum LH and carotid IMT has not been previously studied. To examine the hypothesis that androgen deficiency in aging men is a risk factor for the development of atherosclerosis, we have examined the relationship of andropause with cardiovascular risk factors and carotid IMT in a group of 239 men between 40 and 70 years of age.

METHODS

Subjects. The population from which the subjects were selected consisted of all 40- to 70-year-old men living in the city of Turku, Finland (n = 28,622, year 2000). This cohort received by mail a questionnaire that inquired about general well-being and andropausal symptoms (15,500 men returned the questionnaire). The questionnaire was composed of three parts: 1) the simplified Turku 3-Query (T3Q) Questionnaire (20); 2) the Aging Male Symptoms (AMS) Questionnaire (21); and 3) query of known illnesses and medications. The T3Q Questionnaire had three questions: 1) have you had weakening in tasks requiring strength; 2) decreased libido; or 3) depression during the last five years? Each "yes" answer yielded one point, with a consequent range of scores 0 to 3. The 2,513 men who fulfilled the criteria for significant andropausal symptoms (≥2 points in T3Q) were invited to have serum T and LH measurements taken, and 1,764 (70.2%) participated. Ninety-nine men who fulfilled the criteria for significant andropausal symptoms as well as hormonal criteria of andropause (serum total T <9.8 nmol/l or LH >6 U/l and T >9.8 nmol/l), had no clinical manifestations of CHD, and had not been previously diagnosed with hypertension or diabetes were invited into the study. Serum glycated hemoglobin (HbA_{1c}) was measured in the andropause group, and three andropausal men who had HbA_{1c} exceeding the reference range (4.0% to 6.2%) were excluded from the analyses. In addition, a pilot sample of 140 men (200 were invited, 70% participated) of the cohort who did not fulfill the criteria for andropause (normal T, no significant symptoms) were studied. The testosterone cut-off limit was based on a pilot study of the main study population of the same age. Subjects using any hormones, medication for erectile dysfunction, or coumarin-type anticoagulants were excluded from the study.

The study protocol had been approved by the Joint Committee on Ethics of the University of Turku and the Turku University Central Hospital. All participants gave written, informed consent, and the study was conducted according to the declaration of Helsinki.

Measurement of serum T, LH, sex hormone-binding globulin, and E_2. Venous blood samples were drawn from an antecubital vein in the morning (7:30 to 11:30 AM) after an overnight fast (8 to 11 h). Serum T was measured using coated-tube spectrophotometry (Spectria Testosterone, Orion Diagnostica, Oulunsalo, Finland). The interassay coefficient of variation (CV) was 6.9%, and the reference range for men was 9.8 to 33 nmol/l. Serum LH was measured using an immunofluorometric assay (AutoDelfia, Wallac Ltd., Turku, Finland). The interassay CV of the method was 4.5%, and the reference range was 1.3 to 7.7 IU/l. The free androgen index was defined as 100 × T/sex hormone-binding globulin. Sex hormone-binding globulin was measured using an immunofluorometric assay (AutoDelfia, Wallac Ltd., Turku, Finland), and E_2 using a spectrophotometric assay (Spectria Estradiol, Orion Diagnostica, Oulunsalo, Finland). The interassay CVs of these methods were 3.4% and 10.0%, respectively. None of the men had serum T less than the detection limit of the assay.

Serum lipids. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were measured using standard enzymatic methods, Boehringer Mannheim GmbH reagents, and a fully automated analyzer (Hitachi 917; Hitachi Ltd., Tokyo, Japan). The LDL cholesterol concentration was calculated using the Friedewald equation (22).

Ultrasound studies. All studies were performed by an experienced vascular sonographer who was blinded to the clinical characteristics of the participants, using an Acuson Sequoia 512 mainframe (Acuson, Mountain View, California) with a 13.0-MHz linear-array transducer. The studies were performed on fasting patients between 7:30 and 9:00 AM. To minimize external stimuli, all studies were carried out in a silent clinical research laboratory room. Blood pressure was measured twice during the ultrasound examination from the nondominant arm using a sphygmomanometer. All studies were done following a predetermined, standardized scanning protocol for the right and left carotid arteries. The proximal part of the carotid bulb was identified, and the

Table 1. Characteristics of the Study Groups

	Control Men (n = 140)	Andropausal Men (n = 96)	p Value
Age (yrs)	56.7 ± 8.1	58.3 ± 7.3	0.13
Height (m)	1.78 ± 0.06	1.79 ± 0.07	0.70
Weight (kg)	85.3 ± 11.6	90.3 ± 13.8	0.004
Body mass index (kg/m^2)	27.0 ± 3.1	28.6 ± 3.9	0.001
Systolic blood pressure (mm Hg)	147 ± 19	146 ± 18	0.75
Diastolic blood pressure (mm Hg)	88 ± 11	87 ± 10	0.34
Total cholesterol (mmol/l)	5.72 ± 0.85	5.53 ± 0.85	0.15
LDL cholesterol (mmol/l)	3.63 ± 0.74	3.40 ± 0.77	0.04
HDL cholesterol (mmol/l)	1.44 ± 0.35	1.38 ± 0.36	0.24
Triglycerides (mmol/l)	1.54 ± 1.05	1.66 ± 0.89	0.39
Testosterone (nmol/l)	16.74 ± 4.66	13.00 ± 6.16	0.001
Free androgen index (no unit)	39.9 ± 13.6	33.8 ± 12.3	0.003
Luteinizing hormone (U/l)	3.82 ± 1.20	6.94 ± 4.77	0.001
Sex hormone-binding globulin (nmol/l)	45.4 ± 15.5	40.9 ± 21.4	0.13
Estradiol (nmol/l)	98.1 ± 31.5	85.1 ± 27.5	0.005
AMS score	33.6 ± 10.9	40.8 ± 12.9	0.001
Smokers	75 (54%)	52 (54%)	0.74
Maximum common carotid IMT (mm)	1.00 ± 0.23	1.08 ± 0.34	0.045
Maximum IMT of the carotid bulb (mm)	1.27 ± 0.35	1.44 ± 0.48	0.003
Common carotid diameter (mm)	6.73 ± 0.95	6.75 ± 0.94	0.84

Data are presented as the mean value ± SD.
AMS = Heinemann's Aging Male Symptoms; HDL and LDL = high- and low-density lipoprotein, respectively; IMT = intima-media thickness.

segment of the common carotid artery 1 to 2 cm proximal to the bulb was scanned. The image was focused on the posterior wall, and the resolution box function was used to magnify the arterial far wall. Two angles were used in each case: anterior oblique and lateral. All scans were digitally stored for subsequent off-line analysis. Two end-diastolic frames of the best image quality were selected and analyzed for maximum IMT, and the average reading from these two frames was calculated for both the right and left carotid arteries. Moreover, the maximum IMT of the carotid bulb regions was examined on both sides. The images were analyzed off-line, unaware of the individuals' clinical details. In our laboratory, the between-visit CV of IMT measurements was 6.4%, and the between-observer CV was 5.2% (23).

Statistical methods. The results are expressed as the mean value ± SD. Data on serum triglycerides were skewed toward high values and were therefore analyzed after logarithmic transformation. Comparisons between the groups were conducted by the Student *t* test. Univariate associations between the study variables were analyzed by calculating the Pearson's correlation coefficients. Multivariate analyses were done using the stepwise linear regression technique. The following explanatory variables were included in the analysis: serum T, LH, total cholesterol, age, body mass index, systolic blood pressure, and smoking. All statistical analyses were performed using the SAS statistical analysis system (SAS Institute, Gary, North Carolina).

RESULTS

The characteristics of the study groups are shown in Table 1. Andropausal men had a higher Heinemann's Aging Male Symptoms (AMS) score and body mass index, weighed more, and had lower serum LDL cholesterol and E_2 (Table 1). Carotid IMT was increased in andropausal men compared with controls (Table 1). No other differences were observed between the study groups. Common carotid and carotid bulb IMT were similar in men with absolute and relative T deficiency ($p > 0.24$).

The age-adjusted univariate associations between carotid IMT and risk factors are shown in Table 2. Carotid IMT was inversely associated with serum T levels (Fig. 1) and the HDL/total cholesterol ratio and correlated positively with LH (Fig. 2), age, blood pressure, body mass index, total cholesterol, and LDL cholesterol. In the stepwise multivariate regression model, the independent explanatory variables

Table 2. Univariate and Age-Adjusted Correlates of Carotid Artery Intima-Media Thickness in 236 Men

	Maximum Carotid IMT (r, p value)	Maximum IMT of Carotid Bulb (r, p value)
Age	0.48, 0.001	0.34, 0.001
Body mass index	0.16, 0.02	0.08, 0.26
Systolic blood pressure	0.14, 0.06	0.03, 0.70
Diastolic blood pressure	0.15, 0.04	0.00, 0.98
Total cholesterol	0.25, 0.001	0.13, 0.08
LDL cholesterol	0.25, 0.001	0.18, 0.01
HDL/total cholesterol	−0.21, 0.003	−0.21, 0.004
Triglycerides	0.22, 0.003	−0.04, 0.63
Testosterone	−0.17, 0.02	−0.08, 0.29
Free androgen index	−0.04, 0.60	−0.01, 0.97
Luteinizing hormone	0.20, 0.005	0.25, 0.001
Sex hormone-binding globulin	−0.06, 0.37	−0.05, 0.49
Estradiol	0.04, 0.57	−0.03, 0.67

Abbreviations as in Table 1.

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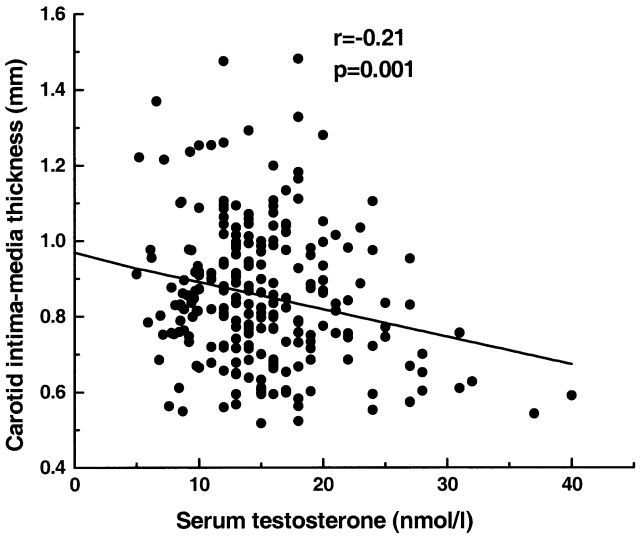

Figure 1. Correlation between serum T and maximum intima-media thickness of the carotid bulb in 236 middle-aged men.

for carotid IMT were age (maximum IMT: p = 0.001; maximum bulb IMT: p = 0.001), total cholesterol (maximum IMT: p = 0.001; maximum bulb IMT: p = 0.022), T (maximum IMT: p = 0.003; maximum bulb IMT: p = 0.039), LH (maximum IMT: p = 0.006; maximum bulb IMT: p = 0.001), and smoking (maximum IMT: p=0.025).

When the group variable for andropause was included in the multivariate model instead of T and LH, the significant explanatory variables for maximum common carotid IMT were the group variable (p = 0.004), age (p = 0.001), total cholesterol (p = 0.001), and smoking (p = 0.031). The free androgen index, when substituted for T, was not associated with IMT in the multivariate model.

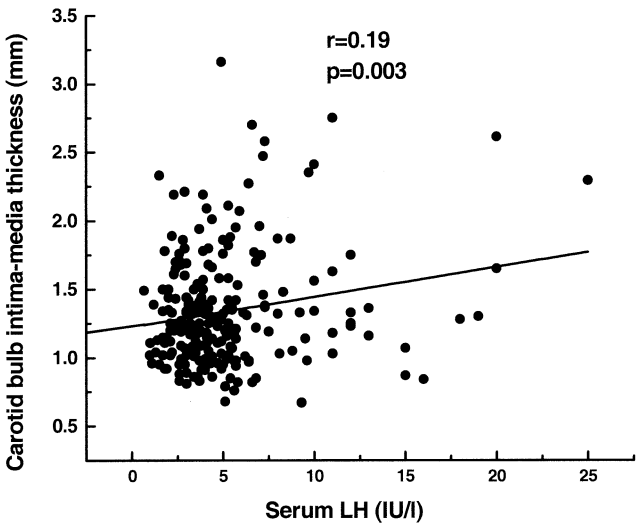

Figure 2. Correlation between serum luteinizing hormone (LH) and maximum intima-media thickness of the carotid bulb in 236 middle-aged men.

DISCUSSION

The present study shows that hypogonadal status (absolute or compensated), together with a high andropausal symptom score, is associated with increased carotid artery IMT, a marker of subclinical atherosclerosis in middle-aged men. Moreover, serum T was inversely and serum LH directly associated with carotid IMT after controlling for CHD risk factors in the multivariate model. The latter finding brings further significance for the age-related increase of LH levels as an indicator of the initial or compensated stage of hypogonadism, as observed previously by our group (11).

The prevalence of CHD shows a consistent male to female ratio of 2.2 (range 1.2 to 4.5) among different populations, despite considerable variations in absolute rates (24). This gender difference has been assumed to be a consequence of atheroprotection exerted by estrogen (25), along with detrimental effects of androgens. In contrast, most (1) but not all (3,26) observational studies in recent years have demonstrated an inverse relationship between endogenous serum T levels and CHD. Furthermore, the data from experimental studies remain inconclusive, as T has been shown to induce both proatherogenic and antiatherogenic effects on the vasculature (27).

Recent studies examining carotid IMT have shown an inverse association between serum T levels in elderly men 74 to 92 years of age (16), in men with type II diabetes (17), and in obese men with glucose intolerance (18). Moreover, Hak et al. (28) demonstrated in a large population-based study that serum T levels inversely and independently correlated with the presence of aortic calcified plaques and the progression of aortic atherosclerosis. Our current findings are in line and extend the findings of these previous studies by demonstrating an inverse association between serum T and IMT in middle-aged men. The causality of this association needs to be examined in prospective studies, but together, the current evidence suggests that normal androgen levels may protect aging men from the development of atherosclerosis. The possible mechanisms may include anti-inflammatory effects of normal physiological levels of sex hormones, regulation of apoptosis, and promotion of smooth muscle cell stability (27).

Experimental male animal studies have shown that androgens reduce diet- and injury-induced atherosclerosis (27). Some studies have implicated that the beneficial effects of T supplementation in T-deficient animal models would be mediated via an increase in E_2 levels due to conversion of T by aromatase (9). In the present study, however, serum E_2 was not associated with carotid IMT. Surprisingly, serum LH levels were associated with IMT also in the multivariate model after controlling for serum T. There are currently no data indicating that common carotid artery endothelial cells express LH receptors, but these receptors have been found in the carotid rete around the pituitary in E_2-pretreated ewes (29) and in human endometrial and myometrial blood vessels (30). Therefore, it should not be assumed that the

association between carotid IMT and serum LH levels is merely a secondary reflection or epiphenomenon of increased serum LH in men with absolute or relative T deficiency. Nevertheless, the association between LH and IMT is interesting and warrants further studies to test the potential causality of this finding.

Andropause was associated with obesity in the current study. Increased fat mass is associated with increased conversion of androgens to estrogens. Furthermore, obesity-associated hyperleptinemia has a suppressive effect on the hypothalamic-pituitary-gonadal axis (31). Previous studies have associated T deficiency (32) with central and upper-body obesity, and low T levels have been shown to precede the development of obesity (33). Obesity is a risk factor for increased IMT (34). However, the association between andropause and serum T and IMT persisted in the multivariate model after controlling for body mass index.

Study limitations. Our study has limitations. The associations between serum T, LH, and carotid atherosclerosis were evaluated in a cross-sectional setting. The study included a relatively limited number of subjects. However, the findings were rather distinct, and the small sample size is unlikely to detract from the validity of the main findings. Instead of free T, we only measured serum total T and sex hormone-binding globulin and calculated from these the free androgen index, which was used as a marker of free T levels. The free androgen index was not associated with IMT in the multivariate model, which was not unexpected, because this parameter has previously been shown to be an unreliable estimate of free T (35). Direct measurement of free T using the equilibrium dialysis technique would, in theory, provide the most reliable estimate of the biologically active T concentration (35). In practice, this method is not only expensive and laborious but also less consistent than the other estimates of T. Therefore, a lack of accurate measure of serum free T concentrations may have had a diluting effect, if any, on our findings concerning the association between serum T levels and IMT.

Conclusions. Our findings demonstrate an association between serum T levels, andropause, and carotid artery IMT in middle-aged men. These results raise the possibility that individuals with andropausal symptoms, together with impaired sex-hormone status and increased carotid IMT, might benefit from T replacement therapy to decelerate the progression of atherosclerosis and protect from its clinical sequelae (i.e., CHD, ischemic stroke, and peripheral vascular disease). The ultrasound measurement of carotid IMT might provide an additional clinical tool for decision-making and CHD risk stratification during consideration of taking up T supplementation in individual patients. It is, however, important to consider the major change in our perception of the effects of hormone replacement therapy on the risk of CHD in women during recent years. Despite the fact that earlier observational studies associated hormone replacement therapy use with a 40% to 50% reduced risk of CHD, the recent randomized clinical trials have demonstrated that hormone replacement therapy does not confer cardiac protection and may even slightly increase the risk of coronary events (36,37). Therefore, randomized, controlled trials are required to establish the effects of T supplementation on the occurrence of CHD in andropausal men before wide scale use of these regimens can be accepted for prevention of atherosclerotic diseases.

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